

I. BEHAVIOR OF A GENERALIZED SYSTEM WITH A NEGATIVE FEEDBACK

Consider a simple linear ordinary differential equations (ODE) system:

$$\begin{aligned}\dot{x} &= S - \alpha x - \beta y \\ \dot{y} &= \gamma x - \delta y\end{aligned}\tag{1}$$

This represents a simple differential equations approximation of a biochemical system with a negative feedback. It captures, however, main aspects of more complex equations systems with a single negative feedback interaction. In this system the molecular species x and y interact in such a way that x is negatively regulated by y , whereas y is positively regulated by x . In addition, self-regulation of x and y is included and considered to be negative. Biochemically speaking, x activates y , whereas y feeds negatively back to x . In addition, x and y are constitutively downregulated, e.g., by degradation. The Greek letters represent non-negative rate constants. The external signal S determines the degree of activation of the entire system.

Treatment of linear ODE systems can be found in most standard differential equations textbooks (for a good example of comprehensive ODE-Based treatment of biological data see L. Edelstein-Koshet, *Mathematical Models in Biology*, McGraw Hill, New York, NY, 1988). The behavior of [1] is determined by the values of parameters: $a = -\alpha - \delta$ and $b = \alpha\delta + \beta\gamma$. We note that we always have: $a \leq 0$ and $b \geq 0$. These conditions lead to two possible “phase portraits” of the system: the stable spiral or the stable node. The stable spiral implies damped oscillations for the values of both x and y when plotted as a function of time as both variables approach the stable steady state determined by S . The stable spiral is actualized if $a^2 < 4b$. In other words, the lower the value of a compared to b , the more “oscillatory” or less damped the behavior. The limiting case of $a = 0$ (corresponding to “center”, see above) generates undamped oscillations. A related result is that the value of a determines how fast the system relaxes to the steady state value, whereas the value of b determines the frequency of the oscillations.

The above treatment interpreted biochemically means that the higher the feedback regulation terms (represented by b) compared to self-regulation terms (represented by a), the less damped (or more pronounced) is the oscillatory response to changes in the external signal S . The frequency of the oscillations is inversely proportional to b . Therefore an increase in system parameters determining b for [1] will strengthen the negative feedback but will also increase the propensity for oscillations in the response. In some biochemical systems with negative feedbacks oscillations can thus arise as a by-product of the necessity of fast signal down-regulation.

II. DESCRIPTION OF THE MODEL

Introduction

The focus of the model is the central signaling module that appears to transduce all NF- κ B responses. It consists of IKK represented here as a single signaling entity (without separate descriptions for IKK1/IKK2 (IKK α /IKK β) heterodimer scaffolded by NEMO (IKK γ)), three mammalian I κ B isoforms (α , β and ϵ), and NF- κ B. NF- κ B heterodimer isoforms are not specified in this model, a simplification that has two justifications: (1) A single NF- κ B isoform (p50/p65) with transcriptional activation potential predominates in many cells. (2) I κ B-NF- κ B interaction appears to be promiscuous with little molecular preferences evident in NF- κ B/Rel or I κ B protein knockouts (A. Hoffmann, unpublished data). While future version of the model may consider the substructure of multi-protein complexes, the present model is adequate in addressing the role of different I κ B isoforms in controlling the dynamic response of the pathway to transient and persistent stimulation.

The model consists of a series of ordinary differential equations describing the time evolution of concentrations of various molecules and molecular complexes of interest. In particular, nuclear NF- κ B, bimolecular IKK-I κ B, I κ B-NF- κ B and trimolecular IKK-I κ B-NF- κ B complexes for each I κ B isoform are explicitly considered. The participating molecular species are assumed to translocate between two subcellular compartments: the cytoplasm and the nucleus, thus necessitating considerations about transport rates in addition to binding and reaction rates. The input into the signaling module is represented by the concentration of the active IKK. This assumption allows the model to be applied to a variety of ligands – different ligands may, however, lead to different IKK activation profiles via signaling modules that are not subject of this study. Further, we assumed that ubiquitination and proteolysis of I κ B isoforms follows immediately the specific phosphorylation event of I κ B by IKK. While we can represent the whole process by a single reaction rate here, future versions of the model will distinguish between these steps as more biochemical data becomes available. Thus the interaction of IKK and I κ B isoforms effectively proceeds as a single enzymatic degradation reaction scheme:



where E is the enzyme, S is the substrate and ES is the enzyme-substrate complex. This reaction scheme gives rise to three differential equations describing the rates of change of the concentrations of E, S and ES.

In the computational model all four rates are described explicitly. In general, enzymes, substrates and products of individual reactions can be shared among multiple reactions giving rise to more complex differential equations for the corresponding concentrations. Thus, in order to describe changes in the concentration of a reaction component completely, all reactions that this component participates in, plus possible transport, degradation and complex formation rates must be considered.

Nomenclature

Uppercase letters denote concentrations of molecular species. All are proteins, except those subscripted with t, which are relevant messenger RNA transcripts.

Lower case letters with numbers represent the corresponding model parameters.

Uncomplexed molecules are described by the designated symbol immediately followed by (t).

Complexes of molecules are described by the designated symbols of their component molecules written next to each other without separation by (t).

The subscripted n indicates that the molecule is in the nucleus.

Free cytoplasmic NF- κ B protein

The first two lines represent the depletion of free NF- κ B due to formation of I κ B-NF- κ B complexes (the terms containing the association rate constants $a4$, $a5$, $a6$) and formation of free NF- κ B due to dissociation of these complexes (the terms containing the dissociation rate constants $d4$, $d5$, $d6$).

The next three lines describe the changes in the concentration of free NF- κ B due to binding to and dissociation from binary IKK-I κ B complexes and freeing of NF- κ B due to I κ B degradation in these complexes following its phosphorylation by IKK (the reaction rate terms are $r4$, $r5$ and $r6$).

The next line describes the rate of degradation of I κ B isoforms in I κ B-NF- κ B complexes (the terms with the degradation rate constant $deg4$ assumed to be equal for all the isoforms).

The final line of the equation represents the first order transport terms for the transport of NF- κ B into (the rate constant $k1$) and out of the nucleus (the rate constant $k01$).

$$\begin{aligned}
 NF-\kappa B'(t) = & -a4 I\kappa B\alpha(t) NF-\kappa B(t) + d4 I\kappa B\alpha NF-\kappa B(t) - a5 I\kappa B\beta(t) NF-\kappa B(t) + d5 \\
 & I\kappa B\beta NF-\kappa B(t) \\
 & -a6 I\kappa B\epsilon(t) NF-\kappa B(t) + d6 I\kappa B\epsilon NF-\kappa B(t) \\
 & -a4 IKK I\kappa B\alpha(t) NF-\kappa B(t) + (r4 + d4) IKK I\kappa B\alpha NF-\kappa B(t) \\
 & -a5 IKK I\kappa B\beta(t) NF-\kappa B(t) + (r5 + d5) IKK I\kappa B\beta NF-\kappa B(t) \\
 & -a6 IKK I\kappa B\epsilon(t) NF-\kappa B(t) + (r6 + d6) IKK I\kappa B\epsilon NF-\kappa B(t) \\
 & + deg4 I\kappa B\alpha NF-\kappa B(t) + deg4 I\kappa B\beta NF-\kappa B(t) + deg4 I\kappa B\epsilon NF-\kappa B(t) \\
 & -k1 NF-\kappa B(t) + k01 NF-\kappa B_n(t),
 \end{aligned}$$

Free nuclear NF- κ B protein

The terms describe transport into and out of the nucleus, and sequestration through nuclear I κ B isoforms.

$$\begin{aligned}
 NF-\kappa B_n'(t) = & k1 NF-\kappa B(t) - a4 I\kappa B\alpha_n(t) NF-\kappa B_n(t) + d4 I\kappa B\alpha_n NF-\kappa B_n(t) - \\
 & a5 I\kappa B\beta_n(t) NF-\kappa B_n(t) + d5 I\kappa B\beta_n NF-\kappa B_n(t) - a6 I\kappa B\epsilon_n(t) NF-\kappa B_n(t) + \\
 & d6 I\kappa B\epsilon_n NF-\kappa B_n(t) - k01 NF-\kappa B_n(t),
 \end{aligned}$$

I κ B mRNA transcript levels

An accurate mathematical account of regulation of transcription and translation is difficult because of inherent complexity of the underlying regulatory processes that are still to a large measure unknown. We have thus given simple semi-qualitative descriptions of the dependence of the rates of transcription and translation on the concentration of NF- κ B and the concentration of I κ B transcripts. We have opted to include separate descriptions of transcription and translation since the data on mRNA concentration as a function of time are increasingly available through DNA microarray and other technologies. The transcription of I κ B isoforms was assumed to be a second order process proportional to the square of nuclear NF- κ B concentration for I κ B α and zero order constitutive processes for all three isoforms. The second order dependence of inducible I κ B α transcription was introduced to capture the fact that NF- κ B is a dimer and as such can be presumed to bind DNA in a non-linear, cooperative fashion. This non-linearity, however, is not essential for the qualitative behavior of the model, as can be easily shown computationally. The degradation of I κ B mRNA transcripts was described according to unsaturable first order kinetics for all isoforms. The sensitivity of the model to variations of the synthesis and degradation rates is examined in Supplement 3 and Fig. 6 of the paper.

$$I\kappa B\alpha_i'(t) = tr2a + tr2 \text{ NF-}\kappa B_n(t)^2 - tr3 \text{ I}\kappa B\alpha_i(t),$$

$$I\kappa B\beta_i'(t) = tr2b - tr3 \text{ I}\kappa B\beta_i(t),$$

$$I\kappa B\epsilon_i'(t) = tr2e - tr3 \text{ I}\kappa B\epsilon_i(t),$$

Free cytoplasmic I κ B proteins

The equations account for cytoplasmic sequestration by IKK (association and dissociation rate constants: a1 and d1 for I κ B α , a2 and d2 for I κ B β and a3 and d3 for I κ B ϵ) and NF- κ B, nuclear transport (with the rate of import tp1 and export tp2 assumed to be equal for all isoforms), synthesis (the rate of translation tr1) and constitutive degradation (deg1 assumed to be equal for all isoforms).

$$I\kappa B\alpha'(t) = -a1 \text{ IKK}(t) \text{ I}\kappa B\alpha(t) + d1 \text{ IKK I}\kappa B\alpha(t) - a4 \text{ I}\kappa B\alpha(t) \text{ NF-}\kappa B(t) + d4 \text{ I}\kappa B\alpha \text{ NF-}\kappa B(t) + tr1 \text{ I}\kappa B\alpha_i(t) - deg1 \text{ I}\kappa B\alpha(t) - tp1 \text{ I}\kappa B\alpha(t) + tp2 \text{ I}\kappa B\alpha_n(t),$$

$$I\kappa B\beta'(t) = -a2 \text{ IKK}(t) \text{ I}\kappa B\beta(t) + d2 \text{ IKK I}\kappa B\beta(t) - a5 \text{ I}\kappa B\beta(t) \text{ NF-}\kappa B(t) + d5 \text{ I}\kappa B\beta \text{ NF-}\kappa B(t) + tr1 \text{ I}\kappa B\beta_i(t) - deg1 \text{ I}\kappa B\beta(t) - 0.5 \text{ tp1 I}\kappa B\beta(t) + 0.5 \text{ tp2 I}\kappa B\beta_n(t),$$

$$I\kappa B\epsilon'(t) = -a3 \text{ IKK}(t) \text{ I}\kappa B\epsilon(t) + d3 \text{ IKK I}\kappa B\epsilon(t) - a6 \text{ I}\kappa B\epsilon(t) \text{ NF-}\kappa B(t) + d6 \text{ I}\kappa B\epsilon \text{ NF-}\kappa B(t) + tr1 \text{ I}\kappa B\epsilon_i(t) - deg1 \text{ I}\kappa B\epsilon(t) - 0.5 \text{ tp1 I}\kappa B\epsilon(t) + 0.5 \text{ tp2 I}\kappa B\epsilon_n(t),$$

Free nuclear IκB proteins

Nuclear concentrations of IκB isoforms are determined by nuclear transport and binding to nuclear NF-κB.

$$I\kappa B\alpha_n'(t) = tp1 \ I\kappa B\alpha(t) - tp2 \ I\kappa B\alpha_n(t) - a4 \ I\kappa B\alpha_n(t) \ NF-\kappa B_n(t) + d4 \ I\kappa B\alpha_n \ NF-\kappa B_n(t),$$

$$I\kappa B\beta_n'(t) = 0.5 \ tp1 \ I\kappa B\beta(t) - 0.5 \ tp2 \ I\kappa B\beta_n(t) - a5 \ I\kappa B\beta_n(t) \ NF-\kappa B_n(t) + d5 \ I\kappa B\beta_n \ NF-\kappa B_n(t),$$

$$I\kappa B\varepsilon_n'(t) = 0.5 \ tp1 \ I\kappa B\varepsilon(t) - 0.5 \ tp2 \ I\kappa B\varepsilon_n'(t) - a6 \ I\kappa B\varepsilon_n(t) \ NF-\kappa B_n(t) + d6 \ I\kappa B\varepsilon_n \ NF-\kappa B_n(t),$$

Cytoplasmic IκB-NF-κB complexes

Their presence is determined by binding rates and constitutive IκB degradation.

$$I\kappa B\alpha \ NF-\kappa B'(t) = a4 \ I\kappa B\alpha(t) \ NF-\kappa B(t) - d4 \ I\kappa B\alpha \ NF-\kappa B(t) - a7 \ IKK(t) \ I\kappa B\alpha \ NF-\kappa B(t) + d1 \ IKK I\kappa B\alpha \ NF-\kappa B(t) + k2 \ I\kappa B\alpha_n \ NF-\kappa B_n(t) - deg4 \ I\kappa B\alpha \ NF-\kappa B(t),$$

$$I\kappa B\beta \ NF-\kappa B'(t) = a5 \ I\kappa B\beta(t) \ NF-\kappa B(t) - d5 \ I\kappa B\beta \ NF-\kappa B(t) - a8 \ IKK(t) \ I\kappa B\beta \ NF-\kappa B(t) + d2 \ IKK I\kappa B\beta \ NF-\kappa B(t) + 0.5 \ k2 \ I\kappa B\beta_n \ NF-\kappa B_n(t) - deg4 \ I\kappa B\beta \ NF-\kappa B(t),$$

$$I\kappa B\varepsilon \ NF-\kappa B'(t) = a6 \ I\kappa B\varepsilon(t) \ NF-\kappa B(t) - d6 \ I\kappa B\varepsilon \ NF-\kappa B(t) - a9 \ IKK(t) \ I\kappa B\varepsilon \ NF-\kappa B(t) + d3 \ IKK I\kappa B\varepsilon \ NF-\kappa B(t) + 0.5 \ k2 \ I\kappa B\varepsilon_n \ NF-\kappa B_n(t) - deg4 \ I\kappa B\varepsilon \ NF-\kappa B(t),$$

Nuclear IκB-NF-κB complexes

The equations above are balanced by the corresponding equations describing the formation of the nuclear IκB-NF-κB complexes. IκBβ-NF-κB and IκBε-NF-κB complexes are transported with the rates equal to 0.5 of the rate of IκBα-NF-κB complexes (tp2). (see Parameter Estimation)

$$I\kappa B\alpha_n \ NF-\kappa B_n'(t) = a4 \ I\kappa B\alpha_n(t) \ NF-\kappa B_n(t) - d4 \ I\kappa B\alpha_n \ NF-\kappa B_n(t) - k2 \ I\kappa B\alpha_n \ NF-\kappa B_n(t),$$

$$I\kappa B\beta_n \ NF-\kappa B_n'(t) = a5 \ I\kappa B\beta_n(t) \ NF-\kappa B_n(t) - d5 \ I\kappa B\beta_n \ NF-\kappa B_n(t) - 0.5 \ k2 \ I\kappa B\beta_n \ NF-\kappa B_n(t),$$

$$I\kappa B\varepsilon_n \ NF-\kappa B_n'(t) = a6 \ I\kappa B\varepsilon_n(t) \ NF-\kappa B_n(t) - d6 \ I\kappa B\varepsilon_n \ NF-\kappa B_n(t) - 0.5 \ k2 \ I\kappa B\varepsilon_n \ NF-\kappa B_n(t),$$

Free cytoplasmic IKK protein

Accounting for adaptation to the signal, binding rates and I κ B degradation due to IKK activity.

$$\begin{aligned} IKK'(t) = & -k_{02} IKK(t) - a_1 IKK(t) I\kappa B\alpha(t) + (d_1 + r_1) IKK I\kappa B\alpha(t) - \\ & a_2 IKK(t) I\kappa B\beta(t) + (d_2 + r_2) IKK I\kappa B\beta(t) - \\ & a_3 IKK(t) I\kappa B\epsilon(t) + (d_3 + r_3) IKK I\kappa B\epsilon(t) - \\ & a_7 IKK(t) I\kappa B\alpha NF-\kappa B(t) + (d_1 + r_4) IKK I\kappa B\alpha NF-\kappa B(t) - \\ & a_8 IKK(t) I\kappa B\beta NF-\kappa B(t) + (d_2 + r_5) IKK I\kappa B\beta NF-\kappa B(t) - \\ & a_9 IKK(t) I\kappa B\epsilon NF-\kappa B(t) + (d_3 + r_6) IKK I\kappa B\epsilon NF-\kappa B(t), \end{aligned}$$

Cytoplasmic IKK-I κ B complexes

Accounting for binding rates and I κ B degradation due to IKK activity.

$$IKK I\kappa B\alpha'(t) = a_1 IKK(t) I\kappa B\alpha(t) - (d_1 + r_1) IKK I\kappa B\alpha(t) - a_4 IKK I\kappa B\alpha(t) NF-\kappa B(t) + d_4 IKK I\kappa B\alpha NF-\kappa B(t),$$

$$IKK I\kappa B\beta'(t) = a_2 IKK(t) I\kappa B\beta(t) - (d_2 + r_2) IKK I\kappa B\beta(t) - a_5 IKK I\kappa B\beta(t) NF-\kappa B(t) + d_5 IKK I\kappa B\beta NF-\kappa B(t),$$

$$IKK I\kappa B\epsilon'(t) = a_3 IKK(t) I\kappa B\epsilon(t) - (d_3 + r_3) IKK I\kappa B\epsilon(t) - a_6 IKK I\kappa B\epsilon(t) NF-\kappa B(t) + d_6 IKK I\kappa B\epsilon NF-\kappa B(t),$$

Cytoplasmic IKK-I κ B-NF- κ B complexes

Accounting for binding rates between IKK-I κ B complexes and NF- κ B, as well as I κ B-NF- κ B complexes and IKK, and I κ B degradation due to IKK activity.

$$IKK I\kappa B\alpha NF-\kappa B'(t) = a_7 IKK(t) I\kappa B\alpha NF-\kappa B(t) + a_4 IKK I\kappa B\alpha(t) NF-\kappa B(t) - (d_1 + d_4 + r_4) IKK I\kappa B\alpha NF-\kappa B(t),$$

$$IKK I\kappa B\beta NF-\kappa B'(t) = a_8 IKK(t) I\kappa B\beta NF-\kappa B(t) + a_5 IKK I\kappa B\beta(t) NF-\kappa B(t) - (d_2 + d_5 + r_5) IKK I\kappa B\beta NF-\kappa B(t),$$

$$IKK I\kappa B\epsilon NF-\kappa B'(t) = a_9 IKK(t) I\kappa B\epsilon NF-\kappa B(t) + a_6 IKK I\kappa B\epsilon(t) NF-\kappa B(t) - (d_3 + d_6 + r_6) IKK I\kappa B\epsilon NF-\kappa B(t),$$

I. PARAMETER VALUES IN THE LITERATURE

1. I κ B mRNA and protein half-lives

The I κ B mRNA half-life is estimated to be 30min. - 2 hrs. (11), which constrains the rate constant for I κ B mRNA transcript degradation to 0.0004 s^{-1} to 0.0001 s^{-1} .

I κ B protein half-life (signal-independent) complexed to NF- κ B: 550 min; half-life of free I κ B: 110 min.(3)
The rate of constitutive I κ B degradation when bound to NF- κ B is $\ln 2 / (550 \times 60 \text{ s}) = 0.00002 \text{ s}^{-1}$
and when not bound to NF- κ B is $\ln 2 / (110 \times 60 \text{ s}) = 0.0001 \text{ s}^{-1}$.

2. Interactions between IKK, I κ B and NF- κ B

IKK-I κ B interaction (in the absence of NF- κ B):

The following data are from (4).

Table 1: Kinetics for the interaction between IKK2 and the I κ B Isoforms

I κ B isoform	$k_a \text{ (M}^{-1} \text{ s}^{-1})$	$k_d \text{ (s}^{-1})$	$K_D^a \text{ (M)} = k_d/k_a$
I κ B α	$22.5 (\pm 2.5) \times 10^3$	$1.25 (\pm 0.15) \times 10^{-3}$	56×10^{-9}
I κ B β 1	$10.5 (\pm 1.5) \times 10^3$	$1.25 (\pm 0.15) \times 10^{-3}$	119×10^{-9}
I κ B β 2	$6.0 (\pm 1.0) \times 10^3$	$1.75 (\pm 0.15) \times 10^{-3}$	292×10^{-9}
I κ B γ	$9.0 (\pm 2.0) \times 10^3$	$1.75 (\pm 0.15) \times 10^{-3}$	194×10^{-9}

Table 2: Kinetic parameters K_M and k_{cat} for the phosphorylation of the I κ B isoforms by IKK

I κ B isoform	$K_M \text{ (M)}$	$k_{cat}^a \text{ (s}^{-1})$	$k_{cat}/K_M^b \text{ (s}^{-1} \text{ M}^{-1})$
I κ B α	$1.7 (\pm 0.6) \times 10^{-6}$	3.7×10^{-2}	22×10^3
I κ B β 1	$3.2 (\pm 1.1) \times 10^{-6}$	3.2×10^{-2}	10×10^3
I κ B β 2	$2.8 (\pm 1.0) \times 10^{-6}$	1.5×10^{-2}	5.4×10^3
I κ B γ	$2.6 (\pm 0.4) \times 10^{-6}$	2.2×10^{-2}	8.5×10^3

It is reported in (5) that $k_{cat}^a \approx 10^{-2} \text{ s}^{-1}$ and $K_M = 0.6\text{-}1 \text{ }\mu\text{M}$ in general agreement with the values above.

These values allow us to deduce rate constants for associations dissociation and catalysis:

I κ B isoform	$k_a \text{ (M}^{-1} \text{ s}^{-1})$	$k_d \text{ (s}^{-1})$	$k_{cat} \text{ (s}^{-1})$
I κ B α	22.5×10^{-3}	1.25×10^{-3}	3.7×10^{-2}
I κ B β	6.0×10^{-3}	1.75×10^{-3}	1.5×10^{-2}
I κ B γ	9.0×10^{-3}	1.75×10^{-3}	2.2×10^{-2}

IKK-I κ B interaction (in the presence of NF- κ B):

The existence of the tripartite IKK-I κ B-NF- κ B complex has been demonstrated in (1).

Zandi et al. report that “Kinetic analysis indicated that in the presence of NF- κ B, the K_m for I κ B phosphorylation by IKK decreased from $2.2 \text{ }\mu\text{M}$ to $1.4 \text{ }\mu\text{M}$ and the relative maximum initial velocity V_{max} (or k_{cat}^a) was increased by a factor of 5.” (2)

Thus we conclude that in the presence of NF- κ B k_{cat} is increased by a factor of 5, and k_a is increased 7-8-fold:

I κ B isoform	k_a ($\mu\text{M}^{-1} \text{s}^{-1}$)	k_d (s^{-1})	k_{cat} (s^{-1})
I κ B α	1.85×10^{-1}	1.25×10^{-3}	1.85×10^{-1}
I κ B β	4.8×10^{-2}	1.75×10^{-3}	7.5×10^{-2}
I κ B γ	7.0×10^{-2}	1.75×10^{-3}	1.1×10^{-1}

I κ B-NF- κ B interactions:

Affinity is estimated as being on the order of 1 nM (6), with the dissociation rate constant assumed to be around 0.001 s^{-1} in (7), and association and dissociation rate constants estimated at $18.4 \mu\text{M}^{-1}\text{min}^{-1}$ and 0.055min^{-1} in (6). There is no data on differential affinity constants for I κ B isoforms.

Though these values are somewhat conflicting, they constrain our parameter fitting to $k_a = 0.3 \mu\text{M}^{-1} \text{s}^{-1} - 1 \mu\text{M}^{-1} \text{s}^{-1}$ and $k_d = 0.0003 \text{ s}^{-1} - 0.001 \text{ s}^{-1}$.

In the model the values of the catalysis rates are all 9-10x smaller than experimental catalysis values due to the assumption that the “catalysis” here includes additional processes of ubiquitination and degradation slowing down the overall reaction

3. Cytoplasmic-Nuclear Transport

The transport characteristics of I κ B and NF- κ B and the concentration of NF- κ B are examined in (7).

While I κ B α -NF- κ B complexes are largely cytoplasmic, free NF- κ B is largely nuclear. I κ B α overexpression studies suggest that the ratio of the transport parameters is $k_{\text{imp}}/k_{\text{exp}} \approx 2$. We assume that to be the same for I κ B β and γ .

The time constant of the nuclear import of I κ B α is estimated to be within the 0.01 - 0.07 min^{-1} range. These observations constrain I κ B import to 1.7 - $11.7 \cdot 10^{-4} \text{ s}^{-1}$ and I κ B export to $0.8 - 5.8 \cdot 10^{-4} \text{ s}^{-1}$.

It is also noted in (7) and (8) that I κ B-NF- κ B complex dissociates such that I κ B and NF- κ B to enter the nucleus independently, which means that nuclear import of the I κ B-NF- κ B complex is zero.

When nuclear export is blocked with the small molecule inhibitor leptomycin B, I κ B α and I κ B β show significantly less nuclear accumulation than I κ B γ (10). To reflect that observation, we multiplied the relevant transport terms by a factors of 0.4 or 0.5 in the equations.

However, upon TNF stimulation newly synthesized I κ B α accumulates in the nucleus, binding NF- κ B-DNA complexes (9). To account for this phenomenon, we have included a term that controls the fraction of I κ B α molecules that are subject to export with respect to time: $\text{fr}'[t] = -0.5/(1+t)^2$, $\text{fr}[0] = 0.5$

In this equation, t is given in minutes and not seconds (whereas all of the parameters are given with a time unit of seconds). It should be pointed out that t is the time that has elapsed since IKK stimulation and that this definition still applies after signal removal. Another form of this equation is $\text{fr}[t] = 0.5/(1+t)$, showing that the fraction of I κ B α capable of nuclear export decreases linearly with time.

II. MODEL FITTING PROCEDURES FOR PARAMETER ESTIMATION

Introduction

Most parameter values in the model have been taken directly from the literature, as described above. Other parameter values are constrained by published results. The parameters that were varied during model fitting to the experimental data in Fig. 2 were the rates of transcription and translation of $\text{NF-}\kappa\text{B}$ isoforms and the rate of $\text{I}\kappa\text{B}\alpha\text{-NF-}\kappa\text{B}$ nuclear export and $\text{NF-}\kappa\text{B}$ nuclear import. Note that, since only one $\text{NF-}\kappa\text{B}$ isoform is present in each knockout cell line, the rates of synthesis of each given isoform can be adjusted independently. The rates of translation were assumed to be equal for all isoforms involved. In the parameter estimation it was determined that an increase in the rates of $\text{NF-}\kappa\text{B}$ isoform transcription could be almost exactly compensated by a proportional decrease in the rate of translation. The translation rate was chosen to correspond to a 3-4 min. time constant.

In adjusting the parameters, it is common to use techniques leading to minimization of the sum of squares of the differences between the predicted and actual responses. Among these methods are the gradient descent method, the Levenberg-Marquardt method, the direct search method of Hooke and Jeeves, etc. (see (12) for extended reference list and discussion). However, given a sufficiently complex biochemical network the performance of these methods can be quite poor (13). In addition, these fitting techniques applied to oscillatory behavior skew towards optimizing frequency matches rather than amplitude and overall qualitative shape of the curve, since a small shift in frequency (and the time of the onset of the oscillations) can lead to a substantial increase in the sum of residuals. On the other hand the fitting process is already significantly constrained by the values for previously published parameters.

Reduced model fitting to knock-out cell line data

We first utilized the steepest gradient descent (GD), genetics algorithm (GA) and random search parameter estimation in the Gepasi version 3.1 software package. Random search parameter estimation yielded the best results among several fitting methods as evidenced by the standard deviation of the estimated parameter values. The most challenging model fitting task represents the oscillatory $\text{NF-}\kappa\text{B}$ activation profile in $\text{I}\kappa\text{B}\alpha\text{-/-I}\kappa\text{B}\beta\text{-/-}$ cells. As shown in Fig.S1, there is satisfactory fit at early time points, but the resulting model fails to describe faithfully the overall shape and frequency of oscillations. In particular, there is a considerable overestimation of the degree of the oscillation damping.

Next, we used the parameter estimates derived from the random method and varied them within the standard deviation limits to improve the qualitative fits regarding frequency and amplitude of oscillations. Such qualitative fitting led to a 6% decrease in the estimate of the transcription rate and about a two-fold decrease in the $\text{I}\kappa\text{B}\alpha\text{-NF-}\kappa\text{B}$ nuclear export rate (the model was rather insensitive to variations of the latter). The estimate for the $\text{NF-}\kappa\text{B}$ nuclear import rate was not readjusted.

Both the $\text{NF-}\kappa\text{B}$ transcription rate and the rate of $\text{NF-}\kappa\text{B}$ -NF- κB nuclear export affect the frequency and the amplitude (the degree of damping) of the oscillations. We have varied these parameters, as described above. Similarly, we studied the behavior of the models, in which only $\text{NF-}\kappa\text{B}$ and $\text{I}\kappa\text{B}\alpha$ are expressed (these “knockout” models are generated by equating the rates of transcription for two $\text{NF-}\kappa\text{B}$ isoforms out of three to zero). These studies resulted in the simulation curves presented in Fig. 2 of the paper.

Wild type cell model fitting

To generate the model of the wild type fibroblast response, we first assumed that we can simply equate the transcription rates for all $\text{NF-}\kappa\text{B}$ isoforms to the values determined in double knockout fitting studies. However, as shown in Fig. 2D, these results failed to agree with the experimental data. The model output was fitted to the wild type cell experimental data by adjusting the transcription rates for $\text{NF-}\kappa\text{B}$ and $\text{I}\kappa\text{B}\alpha$ only. These rates were adjusted down 7-fold to result in good qualitative approximation of the wild type behavior. We concluded that it is likely that there is a cross-regulation between $\text{NF-}\kappa\text{B}$ isoforms leading to compensation of the absence of $\text{NF-}\kappa\text{B}$ isoforms in the knockout lines through up-regulation of the existing

isoform. Such parameter adjustment emphasizes that variation of the rates of synthesis of \square and \square isoforms can lead to significant changes in the signaling characteristics.

Conclusion

The goal of this study is not an exact estimation of biochemical parameters. Parameter sensitivity studies (A.L. unpublished results) can explore how error in the estimates may affect the system's response. Importantly, the conclusions of our study regarding the respective functions of I \square B isoforms, the bimodal signal processing characteristics, and the newly revealed mechanism for specificity in gene expression are not dependent on exact parameter estimates. This also suggests that these conclusions are valid in a variety of cell types, physiological or experimental conditions where parameter values may differ.

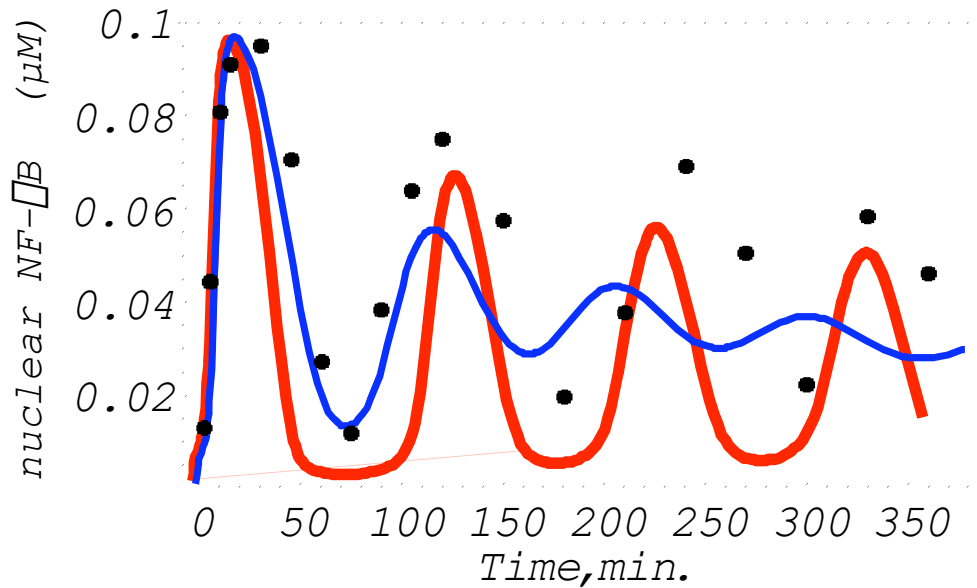


Fig. S1 Comparison of the fitting methods applied to the oscillatory NF- κ B activation profile in I \square B \square ^{-/-} cells. The experimental data are shown as black filled circles, the “semi-quantitative” fit is shown in red and the result of random search fitting in blue. The fitting was performed using the fitting engines supplied with the Gepasi package, version 3.1 (11).

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III. SUMMARY OF PARAMETER VALUES

We have indicated by **black color** the values that were previously determined experimentally, and by **blue color** the values that were constrained by published results. Values in **red color** were derived de novo during model fitting to our experimental data from cells harboring genetically reduced and wild-type I κ B-NF- κ B signaling modules.

Two component interactions:	Symbol:	Values:	Units:
I κ B α -NF- κ B association	a4	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
I κ B α -NF- κ B dissociation	d4	0.5×10^{-3}	s^{-1}
I κ B β -NF- κ B association	a5	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
I κ B β -NF- κ B dissociation	d5	0.5×10^{-3}	s^{-1}
I κ B γ -NF- κ B association	a6	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
I κ B γ -NF- κ B dissociation	d6	0.5×10^{-3}	s^{-1}
IKK-I κ B α association	a1	22.5×10^{-3}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B α dissociation	d1	1.25×10^{-3}	s^{-1}
IKK-I κ B α catalysis	r1	4.07×10^{-3}	s^{-1}
IKK-I κ B β association	a2	6.0×10^{-3}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B β dissociation	d2	1.75×10^{-3}	s^{-1}
IKK-I κ B β catalysis	r2	1.5×10^{-3}	s^{-1}
IKK-I κ B γ association	a3	9.0×10^{-3}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B γ dissociation	d3	1.75×10^{-3}	s^{-1}
IKK-I κ B γ catalysis	r3	2.2×10^{-3}	s^{-1}

Three component interactions:	Symbol:	Values:	Units:
IKK-I κ B α -NF- κ B association	a7	1.85×10^{-1}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B α -NF- κ B dissociation	d1	1.25×10^{-3}	s^{-1}
IKK-I κ B β -NF- κ B association	a4	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B β -NF- κ B dissociation	d4	0.5×10^{-3}	s^{-1}
IKK-I κ B β -NF- κ B catalysis	r4	2.04×10^{-2}	s^{-1}
IKK-I κ B γ -NF- κ B association	a8	4.8×10^{-2}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B γ -NF- κ B dissociation	d2	1.75×10^{-3}	s^{-1}
IKK-I κ B γ -NF- κ B association	a5	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B γ -NF- κ B dissociation	d5	0.5×10^{-3}	s^{-1}
IKK-I κ B γ -NF- κ B catalysis	r5	7.5×10^{-3}	s^{-1}
IKK-I κ B α -NF- κ B association	a9	7.0×10^{-2}	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B α -NF- κ B dissociation	d3	1.75×10^{-3}	s^{-1}
IKK-I κ B β -NF- κ B association	a6	0.5×10^0	$\mu\text{M}^{-1} \text{s}^{-1}$
IKK-I κ B β -NF- κ B dissociation	d6	0.5×10^{-3}	s^{-1}
IKK-I κ B β -NF- κ B catalysis	r6	1.1×10^{-2}	s^{-1}

Synthesis and degradation:	Symbol:	Values:	Units:
I κ B α inducible mRNA synthesis	tr2	1.65×10^{-2}	$\mu\text{M}^{-1} \text{s}^{-1}$
I κ B α constitutive mRNA synthesis	tr2a	1.54×10^{-6}	$\mu\text{M} \text{s}^{-1}$
I κ B α constitutive mRNA synthesis	tr2b*	1.78×10^{-7}	$\mu\text{M} \text{s}^{-1}$
I κ B α constitutive mRNA synthesis	tr2e*	1.27×10^{-7}	$\mu\text{M} \text{s}^{-1}$
I κ B mRNA degradation	tr3:	2.8×10^{-4}	s^{-1}
constitutive I κ B translation rate	tr1	4.08×10^{-3}	s^{-1}
constitutive I κ B degradation (free)	deg1	1.13×10^{-4}	s^{-1}
constitutive I κ B degradation (complexed to NF- κ B)	deg4	2.25×10^{-5}	s^{-1}

* This rate should be multiplied by 7 in the models of κ -/-, κ /-, as described in the text

Nucleo-cytoplasmic transport:	Symbol:	Values:	Units:
I κ B α nuclear import	tp1	3×10^{-4}	s^{-1}
I κ B α nuclear export	tp2	2×10^{-4}	s^{-1}
I κ B α nuclear import	0.5 tp1	1.5×10^{-4}	s^{-1}
I κ B α nuclear export $f_{r'}[t] = -.5 / (1+t)^2$	0.5 tp2	1×10^{-4}	s^{-1}
I κ B α nuclear import	0.5 tp1	1.5×10^{-4}	s^{-1}
I κ B α nuclear export	0.5 tp2	1×10^{-4}	s^{-1}
NF- κ B nuclear import	k1	0.9×10^{-1}	s^{-1}
NF- κ B nuclear export	k01	0.8×10^{-4}	s^{-1}
I κ B α -NF- κ B nuclear export	k2	1.38×10^{-2}	s^{-1}
I κ B α -NF- κ B nuclear export $f_{r'}[t] = -.5 / (1+t)^2$	0.5 k2	5.2×10^{-3}	s^{-1}
I κ B α -NF- κ B nuclear export	0.5 k2	5.2×10^{-3}	s^{-1}

Simulation protocol

As we are considering “active” IKK, all simulations are started with IKK concentration equal to zero. Following “equilibration” for 2000 min., IKK is raised as a step function to $0.1 \mu\text{M}$. The initial concentration of the total NF- κ B is $0.1 \mu\text{M}$ and the initial concentrations of other molecules are zero at the beginning of the equilibration phase (first 2000 min.). We assume that following the signal onset there is a slow adaptation ($k_{02} = 1.2 \cdot 10^{-4} \text{s}^{-1}$; half-life of 2.3 hrs.), gradually reducing active IKK concentration. Signal removal (corresponding to experimental wash of cells to remove TNF) is modeled as adjustment of the adaptation coefficient k_{02} to 0.18s^{-1} (half-life of 5 min.).

Note:

The concentrations of nuclear I κ B α -NF- κ B and nuclear NF- κ B are summed when plotted in NF- κ Bn graphs since both have similar transcriptional capacity; we assume in accordance with (Suyang,H., Phillips,R., Douglas,I., Ghosh,S. 1996 Role of unphosphorylated, newly synthesized I κ B α in persistent activation of NF- κ B. MCB.16:5444-5449.) that the I κ B α -NF- κ B complex containing newly synthesized I κ B α is capable of DNA binding and transcriptional activation and is thus “active” in the model.

BIOCHEMICAL AND MOLECULAR BIOLOGICAL METHODS

Cell lines and cell culture

Human JURKAT and U937 cell lines were grown in DMEM with 10% fetal calf serum and stimulated with 10ng/ml human TNF α (Biogen). Immortalized fibroblast cell lines were made from E14.5 embryos according to the 3T3 protocol in 10% calf serum (S.A. Aaronson and G.J. Todaro 1968, J. Cell. Physiol. vol. 72, pp. 141-148) and maintained in the same manner. Subconfluent or confluent-and-starved (0.5% serum) cells in 10cm polylysine-coated dishes were stimulated with 10ng/ml murine TNF α (Roche).

Electrophoretic Mobility Shift Assay (EMSA)

Following a wash with ice cold phosphate buffered saline (PBS) supplemented with 1mM EDTA, cells were collected (scraped with a rubber policeman, if necessary) into a microfuge tube and pelleted at 2000g. Cells (about 10^6) were resuspended and allowed to swell in 100 μ l CE buffer (10mM Hepes-KOH pH7.9, 60mM KCl, 1mM EDTA, 0.5% NP-40, 1mM DTT, 1mM PMSF) and vortexed for lysis. Nuclei were pelleted at 4000g, and cytoplasmic lysate removed for SDS-PAGE (following protein concentration normalization) and standard Western blot analysis. Antibodies from Santa Cruz Biotechnology, Inc were used for I κ B α (sc-371) and I κ B β (sc-945). A mouse polyclonal antibody was prepared against full-length recombinant mouse I κ B ϵ protein according to standard procedures. For EMSA, Nuclei were resuspended in 30 μ l NE buffer (250mM Tris pH7.8, 60mM KCl, 1mM EDTA, 1mM DTT, 1mM PMSF) and lysed by 3 freeze-thaw cycles. Nuclear lysates are cleared by 14000g centrifugation and adjusted to 1 μ g/ μ l total protein concentration following Bradford assay. 2 μ g of total nuclear protein was reacted at room temperature for 15min. to 0.1pmoles of P32-labelled 30pb double-stranded oligonucleotide containing a consensus κ B-site (AGCTTGCTACAAGGGACTTTCCGCTGTCTACTTT) in 6 μ l binding buffer (10mM Tris.Cl pH7.5, 50mM NaCl, 10% glycerol, 1% NP-40, 1mM EDTA, 0.1 μ g/ μ l polydIdC). Complexes were resolved on a non-denaturing 5% acrylamide (30:0.8) gel containing 5% glycerol and 1x TGE (24.8mM Tris, 190mM glycine, 1mM EDTA) and visualized by standard autoradiography and/or quantitated using a Phosphorimager (Molecular Dynamics), in which the unbound probe (>20 fold excess) was used to normalize for loading variability. Graphed data is 1000x(signal-background)/(unbound probe-background).

RNase Protection Assay (RPA)

Total RNA was made from confluent and starved fibroblasts using Tri-Reagent (Molecular Research Center, Inc). RNase Protection Analysis was performed with 5 μ g RNA using Riboquant probe set mCK-5 (Pharmingen) according to manufacturers instructions.

Generation of the $\text{IkB}\epsilon^{-/-}$ mouse.

The targeting construct results in a deletion of Amino acids 145-343 of $\text{IkB}\epsilon$ and hence disruption of all 6 ankyrin repeats. Standard methods were utilized in the ES cell selection, the deletion blastocyst injections and derivation of a knock-out mouse strain. The targeting construct was generated as follows:

1. Two overlapping clones spanning approximately 20 kb of 129SvJ genomic DNA and containing the entire $\text{IkB}\epsilon$ coding sequence were subcloned from lambda FIXII (Stratagene) into pBSK(+). These subclones, referred to as 8A21H and 10A44, were extensively mapped by restriction digestion and oligonucleotide hybridization.
2. A 2.0 kb Bgl2-Xho1 fragment from 8A21H that contained the first coding exon of $\text{IkB}\epsilon$ was blunted with Klenow polymerase and gel isolated. The fragment was then ligated to a Pme1-digested knockout vector, pLNTKP (kindly provided by F. Alt). This yielded a cloning intermediate called pEK1. The presence of $\text{IkB}\epsilon$ coding nucleotides 1-345 just upstream of the neomycin resistance cassette, at the (Xho1)-vector junction of pEK1, was confirmed by DNA sequencing.
3. Oligonucleotide linkers containing a Xho1 recognition site were ligated to a 7.3 kb Bgl2 fragment of subclone 10A44 ; the fragment was gel purified and cut with Xho1. This fragment was then ligated to pEK1 DNA previously treated with Xho1 and alkaline phosphatase to give a final knockout construct, pEK7.
4. DNA sequencing of pEK7, beginning in the neomycin resistance cassette and extending downstream into genomic DNA, revealed an exon composed of coding nucleotides 1029-1102 of $\text{IkB}\epsilon$. The intron/exon sequence of the deleted portion of genomic DNA was not determined. Therefore, intervening exons are not indicated in the accompanying diagram.
5. The pEK7 construct was linearized with Pac1 and introduced into E14 ES cells by standard methods. A hybridization probe derived from an upstream Bgl2-EcoRV fragment was used to screen HindIII-digested DNA from neo-resistant ES cell colonies as indicated in the accompanying diagram.

$\text{IkB}\epsilon$ targeted deletion

